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13. ABSTRACT (Maximum 200 Words)

The aim of this proposal is to isolate and characterize prostatic epithelial stem cells. This will be done as stem cell and tumor cell biology may be closely linked and stem cells may have a role in the etiology of cancer. Stem cells and tumor cells have many common features including self-renewal, multi-drug resistance, telomerase expression and, in the instance of the prostate, androgen independence. Stem cells from a variety of tissues have been shown to actively efflux the Hoechst dye 33342 through the activity of multi-drugresistance-like proteins and this property has been used to characterize and isolate them as a side population (SP). Our results show that the dorsal prostate contains a small population of cells (0.65%) capable of effluxing the Hoechst dye by an active process (the side population), indicating that cells with features of stem cells are found in the prostate. In addition we show that 96% of the SP cells express Sca-1, a protein expressed by stem cells of other origins. Most of the Sca-1 expressing cells also express (6 integrin which is also found on many primitive cell types. We also show that SP cells have a greater proliferative capacity than non-SP cells as expected if SP cells are of more primitive origin than non-SP cells.

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Table of Contents

Introduction

The aim of this proposal is to isolate and characterize prostatic epithelial stem cells. This will be done as stem cell and tumor cell biology may be closely linked and stem cells may have a role in the etiology of cancer [1-4]. Stem cells and tumor cells have many common features including self-renewal, multi-drug resistance, telomerase expression and, in the instance of the prostate, androgen independence. Stem cells from a variety of tissues have been shown to actively efflux the Hoechst dye [5] through the activity of multi-drug-resistance (MDR)-like proteins [6] and this property has been used to characterize and isolate them as a side population (SP).

There is also a compelling body of evidence that indicates that adult bone marrow (BM)-derived stem cells are able to differentiate into numerous types of tissues [7]. Recent evidence also indicates that gastric cancer can originate from BM-derived cells [8]. This data indicates that BM gives rise not only to normal differentiated cells in a variety of organs but that BM cells are also capable of being transformed within an organ and form tumors.

The aim of this proposal is to determine if cells with properties expressed by side population cells are located in the prostatic tissue of mice, to examine these cells for the expression of marker proteins and to determine if they have growth properties of stem cells. In addition we will determine if BM cells are able to 'home' to prostate and differentiate into tissues of the urogenital tract.

Body

Tasks 1 and 2. To identify and isolate the side population (SP) of Hoechst-effluxing cells from the dorsal prostate (DP) and to identify the proteins expressed in SP cells.

A considerable amount of time and effort was expended in determining the correct conditions for obtaining and isolating a viable SP from mouse prostate. SP cells are able to efflux the Hoechst dye via a MDR-like transporter protein which is an active biological process. Verapamil (50 μM) was added to some tubes to verify the presence of a SP capable of pumping out the Hoechst dye as it inhibits the activity of MDR-like proteins. We found that verapamil addition prevented the appearance of SP cells indicating that this was an active process. The Hoechst concentration, staining time and temperature are critical for obtaining cells with SP properties that are viable. Different tissues require different conditions of labeling and the time of incubation with the Hoechst dye was varied to determination the best conditions for identifying and isolating the SP. As the Hoechst dye is toxic to cells and as viable cells are required for our in vitro studies we determined the conditions that gave us the best SP with the least toxicity. Propidium iodide (PI) at 2μg/ml was added to the cells to identify dead cells as dead cells take up this dye.

Mouse dorsal prostate tissue was digested in collagenase (0.5 mg/ml) for 2 hour followed by digestion in 0.25% trypsin for 10 minutes. We determined that the optimum

conditions for revealing a viable SP in prostate cell digests resulted from incubating cells in Hoechst 33342 (5 μg/ml) for 90 minutes at 37•C. SP cells were evident in the proximal and in the remaining regions of prostates ducts. We also examined the SP for evidence of two proteins known to be expressed by primitive cells - α6 integrin and Sca-1.

The results of a SP experiment are shown in Fig. 1. In order to obtain valid data a considerable number of cells need to be examined as the SP is such a small population. Propidium iodide was added before FACS analysis to eliminate dead cells from the study. After Hoechst incubation 40% of the cells were viable. Fig.1A shows the side scatter (SSC) and forward scatter (FCS) properties (i.e., the size and granularity of the cells) of the viable Hoechst treated cell population. Fig. 1B shows the Hoechst excluding SP (R2) is 0.65% of the viable population (R1) (1036 SP cells from 159,679 viable cells in R1). The expression of α6 integrin and Sca-1 was determined on these cells after incubating the Hoechst treated cells with either PE-labeled (Sca-1) or FITC-labeled (α6 integrin) antibodies to these antigens. Isoytpe matched control antibodies were added to some cells as controls (Fig. 1C). Fig. 1D indicates that 87.9% of the cells in the SP expressed both Sca-1 and α6 integrin whereas 8.1% of SP cells expressed Sca-1 only. We have previously shown that the proximal region of mouse prostatic ducts (which contains the cells with greatest proliferative potential [9]) is enriched in Sca-1 expressing cells. We next examined the SSC and FSC properties of the SP to determine the sizes and granularity of these cells and found that the SP is comprised of cells that are small to medium sized with little granularity (Fig.1E).

These results show that the dorsal prostate contains cells capable of effluxing the Hoechst 33342 dye by an active process (the side population), indicating that cells with features of stem cells are found in the prostate gland. In addition we show that 96% of the SP cells express Sca-1, a protein shown to be expressed by stem cells of other origins [10, 11]. Most of the Sca-1 expressing cells also express α6 integrin which is also found on many primitive cell types [12-14].

Task 3. To determine the growth potential of SP and non-SP cells

We next examined the growth properties of the FACS sorted SP cells by suspending them in collagen gels and determining the numbers and sizes of prostatic ducts formed after 2 weeks incubation at 37°C. We have previously found that primitive prostatic cells have an increased duct-forming efficiency and also form larger and more complex ducts [9]. We found that SP cells formed 21.3 ± 3.8 ducts compared with 6.3 ± 0.6 ducts for non-SP cells ($p < 0.001$; Fig. 2A). The sizes and complexity of the ducts formed from SP cells was also greater than those of non-SP cells (Fig. 2B, C).

These data indicate that SP cells have a greater proliferative capacity than non-SP cells as would be expected if SP cells are of more primitive origin than non-SP cells.

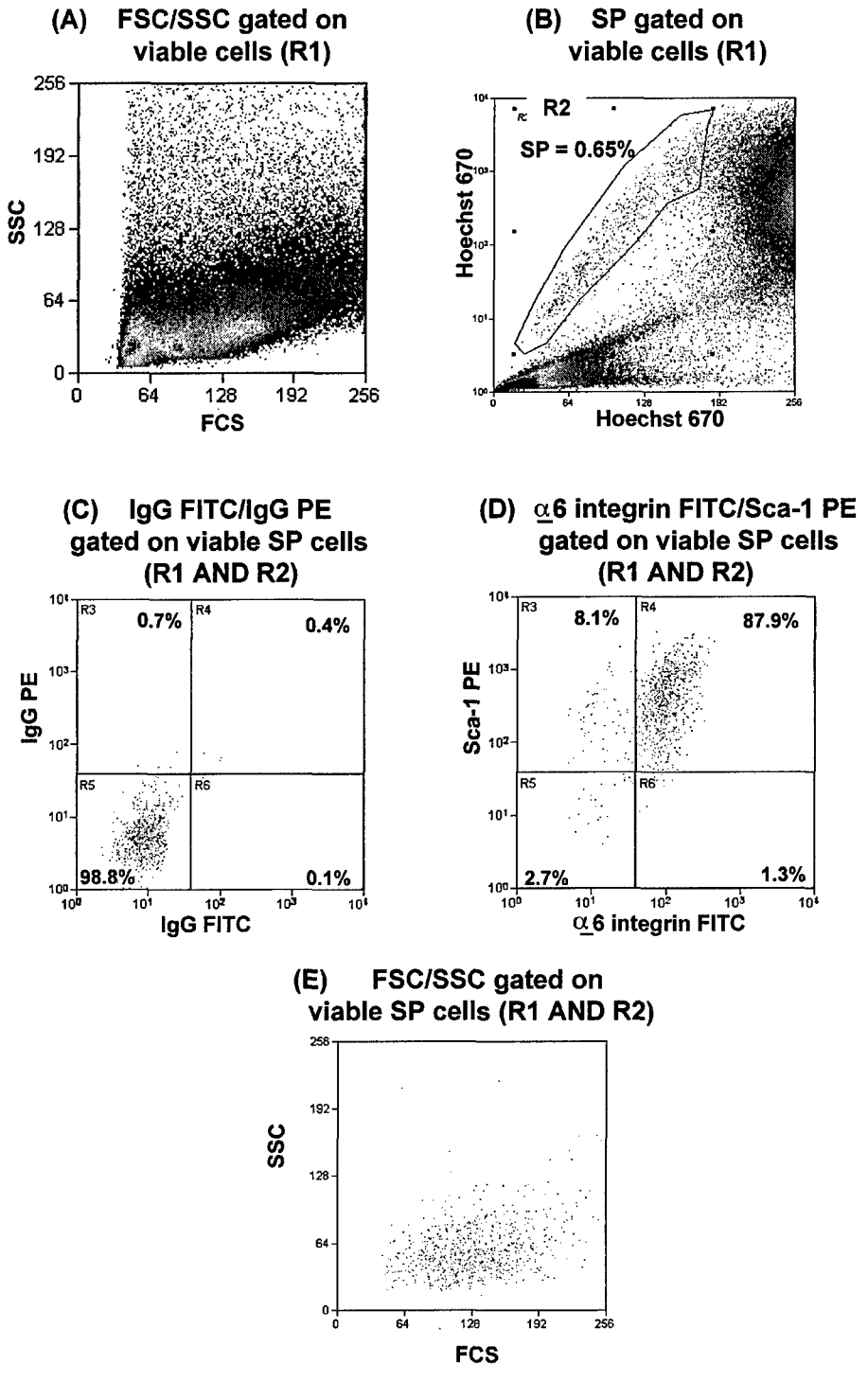

Fig. 1. Dorsal prostate contains SP cells that express Sca-1 and α6 integrin.
A. SSC and FCS properties of the viable Hoechst stained population.
B. Viable SP cells represent 0.65% of the total population. **C.** Hoechst stained cells were incubated with isotype-matched control PE and FITC labeled antibodies. **D.** SP cells express both Sca-1 and α6 integrin. **E.** SSC and FSC properties of the SP cells.

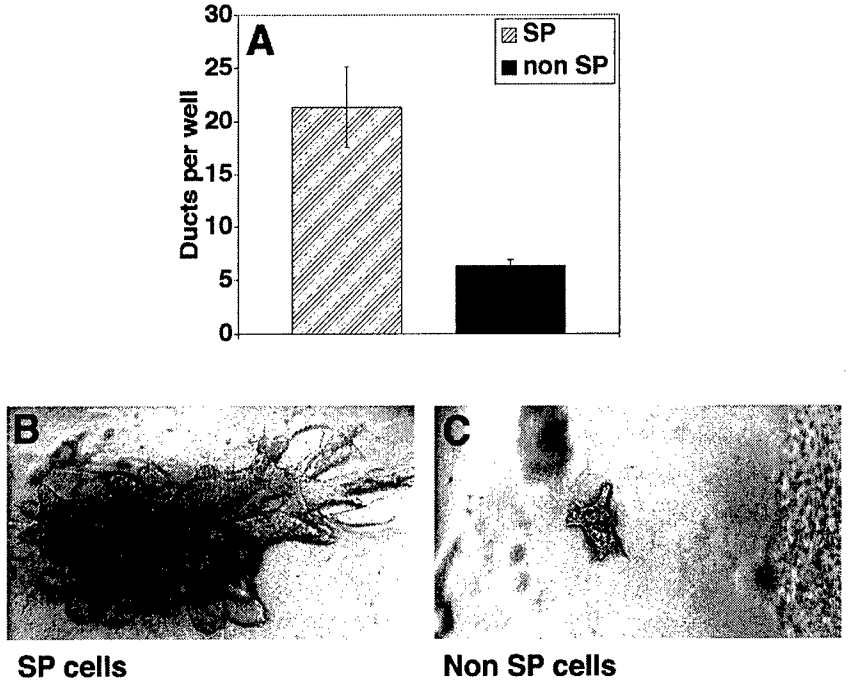

Fig 2. SP cells have greater proliferative potential than non-SP cells.
A. FACS sorted SP and non-SP cells (4000/well) were seeded in collagen gels and the numbers and sizes of ducts were determined after 14 days.
B and C. SP cells (B) formed larger and more complex structures in collagen gels than non-SP cells (C).

Key research accomplishments

1) Conditions for obtaining a Hoechst 33342 dye excluding population of viable prostate stem cells (a side population of prostatic stem cells) have been defined.
2) The prostate SP also expresses two antigens found on stem cells of other origins, namely Sca-1 and α6 integrin.
3) SP cells have greater proliferative potential than non-SP cells in collagen gel assays designed to measure the numbers and sizes of prostatic ducts that arise from individual cells.

Reportable outcomes

We have developed conditions that result in the ability to obtain a viable FACS sorted population of SP from the dorsal prostate of the mouse. This will enable investigators in the field to isolate and study this population of stem/progenitor cells.

Results of these studies will be presented at an NIH meeting on stem cell niches in May 2005.

Conclusions

We have identified a population of prostatic cells capable of effluxing the Hoechst 33342 dye, through an active process. These cells represent 0.65% of the prostatic cell population and are identified as a side population (SP) using FACS analysis. SP cells haven shown to identify a stem cell population in other organs. The prostatic SP cells have greater growth potential in vitro than non-SP cells indicating that they are of a more primitive nature than non-SP cells.

We believe that prostate stem cells and tumor cells are closely linked and expect that prostate tumor cells will have a similar phenotype to normal prostate stem cells. Prostate carcinoma usually progresses to an androgen-independent tumor that may reflect a stem cell-like phenotype. The isolation and elucidation of the phenotype of prostate stem cells is therefore likely to increase our understanding not only of normal prostate physiology but also of prostate carcinoma. The identification of prostate stem cells may also permit the development of new rational therapies to treat prostate carcinoma based on a stem cell phenotype. As normal prostate stem cells and indeed the presence of a prostate are not essential to health, the ablation of normal prostate cells in conjunction with carcinoma cells would have no adverse effects on the patient. Our goal is to elucidate the phenotype of normal prostate stem cells and apply what we learn to prostate carcinoma with the aim of developing new therapies to treat this disease.

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Sandra Coetzee Research Associate Effort 100% On grant until 30 November 2004
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